Preclinical Development of LYL119, a ROR1-Targeted CAR T-Cell Product Candidate Incorporating Four Novel T-Cell Reprogramming Technologies to Overcome Barriers to Effective Cell Therapy for Solid Tumors

Abstract 278

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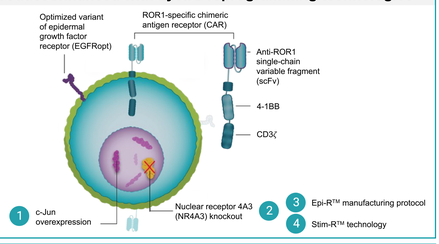
Background

- T-cell exhaustion and lack of durable stemness (defined as the ability of cells to proliferate, persist, and self-renew) are key barriers to effective T-cell therapy in solid tumors[1-2]
- Lyell has developed multiple genetic and epigenetic T-cell reprogramming strategies to overcome these barriers:
  1. Genetically reprogramming T cells through c-Jun overexpression delays exhaustion and results in increased proliferation, sustained cytokine production, and durable antitumor activity[1,3–4]
  2. Genetically reprogramming T cells through *NR4A3* gene KO in combination with c-Jun overexpression further enhances resistance to exhaustion and improves antitumor activity[5]
  3. Epigenetic reprogramming with Lyell's Epi-R™ manufacturing protocol preserves stem-like qualities by controlling T-cell proliferation and differentiation with optimized proprietary cell culture media and other manufacturing steps[1,6–8]
  4. Epigenetic reprogramming with Lyell's Stim-R™ technology (a synthetic biomimetic designed to precisely and physiologically present T-cell activation signals during manufacturing) further improves T-cell polyfunctionality, persistence, and antitumor activity[9]
- These four T-cell reprogramming technologies are combined in LYL119, an investigational ROR1-targeted CAR T-cell product enhanced with c-Jun overexpression, *NR4A3* KO, and Epi-R and Stim-R technologies to overcome barriers to successful T-cell therapy in solid tumors (**Figure 1**)

Figure 1: LYL119, an investigational ROR1-targeted CAR T-cell product enhanced with Lyell's reprogramming technologies

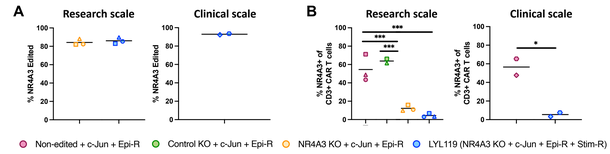


Results

High *NR4A3* genomic editing results in significantly reduced NR4A3 protein expression

- *NR4A3* editing efficiency was similar between NR4A3 KO ROR1 CAR T cells manufactured with or without Stim-R technology and between research and clinical-scale products (**Figure 2**)
- NR4A3 protein reduction in LYL119 was similar between production scales (**Figure 2**)

Figure 2: LYL119 demonstrates high *NR4A3* editing resulting in reduced NR4A3 protein expression

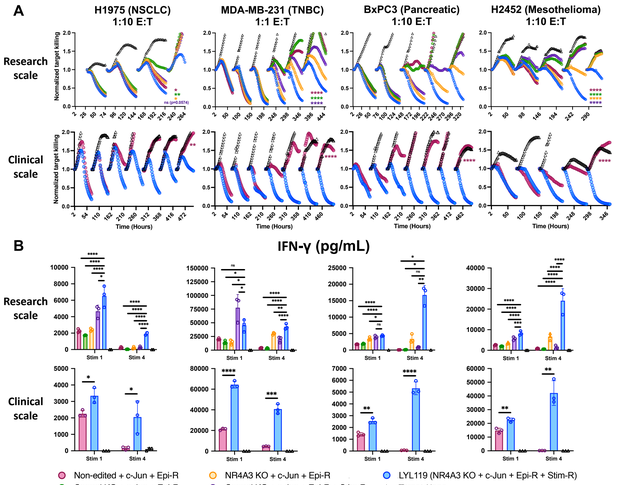


NR4A3 genomic editing efficiency (A) and NR4A3 protein expression (B) in edited ROR1 CAR T cells. Symbols correspond to ROR1 CAR T cells derived from independent donors (n=3 for research and n=2 for clinical scale). Asterisks indicate significant differences. *p<0.05; ***p<0.001 by Tukey's ordinary one-way ANOVA statistical analysis (research) or an unpaired t-test (clinical).

LYL119 demonstrates superior in vitro activity compared to ROR1 CAR T cells lacking one or more reprogramming technologies

Research and clinical-scale LYL119 demonstrated prolonged cytotoxicity and enhanced IFN-γ secretion upon antigen restimulation with multiple different ROR1-expressing solid tumor cell lines (**Figure 3**)

Figure 3: LYL119 demonstrates superior cytotoxicity and enhanced IFN-γ secretion



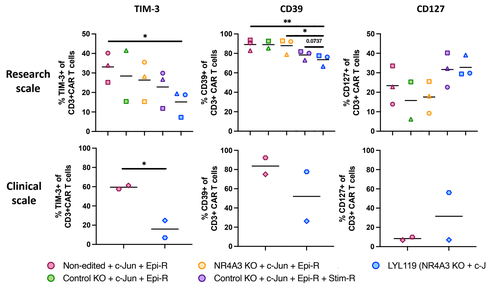
(A) Normalized target killing following sequential stimulation with ROR1+ solid tumor cell lines. Lysis of NucLight Red (NLR)-expressing tumor cells was quantified by measuring total NLR intensity and normalized relative to the starting intensity for each round of stimulation. (B) IFN-γ secretion during sequential exposure to antigen described in (A). Error bars represent mean ± SD of triplicate wells. One representative donor is shown at research (n=3) and clinical (n=2) scale. Asterisks indicate significant differences compared to LYL119 (last timepoint of the last stimulation in A). ns-not significant, *p<0.05; **p<0.005; ***p<0.001; ****p<0.0001 by Tukey's ordinary one-way ANOVA statistical analysis.

LYL119 exhibits an improved phenotype following antigen restimulation

Compared to non-edited ROR1 CAR T cells with c-Jun overexpression alone, LYL119 displayed:

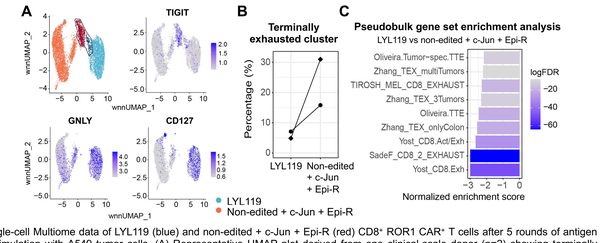
- Further reduction of T-cell exhaustion – Lower TIM-3 and CD39 expression (**Figure 4**), lower proportion of terminally exhausted cluster and significant down-regulation of multiple exhaustion-associated gene sets (**Figure 5**)
- Elevated CD127 expression (**Figure 4**, **Figure 5**), a marker for memory-like T cells, suggesting maintenance of stem-like characteristics
- Global up-regulation of GNLY, an effector-associated marker, suggesting enhanced effector-like characteristics (**Figure 5**)

Figure 4: LYL119 displays reduced T-cell exhaustion and enhanced memory-like phenotype following antigen restimulation



TIM-3, CD39, and CD127 expression after 4 rounds of restimulation with H1975 tumor cells. Symbols correspond to ROR1 CAR T cells derived from independent donors (n=3 for research and n=2 for clinical scale). Asterisks indicate significant differences compared to LYL119. *p<0.05; **p<0.005 by Tukey's ordinary one-way ANOVA statistical analysis (research) or an unpaired t-test (clinical).

Figure 5: LYL119 exhibits reduced T-cell exhaustion and enhanced memory-like and effector-like transcriptomic signatures after antigen restimulation



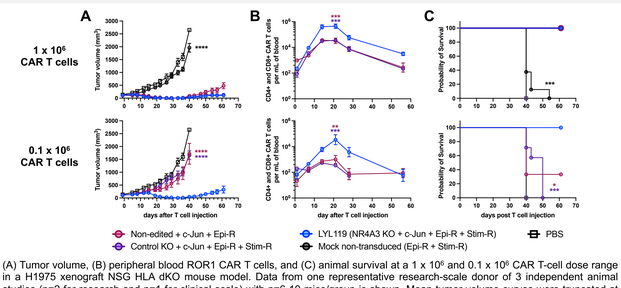
Single-cell Multiome data of LYL119 (blue) and non-edited + c-Jun + Epi-R (red) CD8+ ROR1 CAR+ T cells after 5 rounds of antigen restimulation with A549 tumor cells. (A) Representative UMAP plot derived from one clinical-scale donor (n=2) showing terminally exhausted clusters (circled and in dark blue for LYL119 and dark red for non-edited + c-Jun + Epi-R CAR T cells) enriched for TIGIT RNA expression. LYL119 shows enriched GNLY and CD127 RNA expressions. (B) Percentage of terminally exhausted cluster shown in A. Symbols correspond to CAR T cells derived from independent donors. (C) Exhaustion-associated gene set enrichment analysis.

LYL119 has potent antitumor activity in vivo

Compared to non-edited ROR1 CAR T cells with c-Jun overexpression alone, LYL119:

- Demonstrated robust antitumor activity across a 10-fold CAR T-cell dose range
- Exhibited superior CAR T-cell expansion in the peripheral blood ranging 13- to 62-fold higher on Day 21 after T-cell injection
- Significantly improved animal survival at the low 0.1 x 10^6 CAR T-cell dose (**Figure 6**)

Figure 6: LYL119 demonstrates robust antitumor activity in vivo

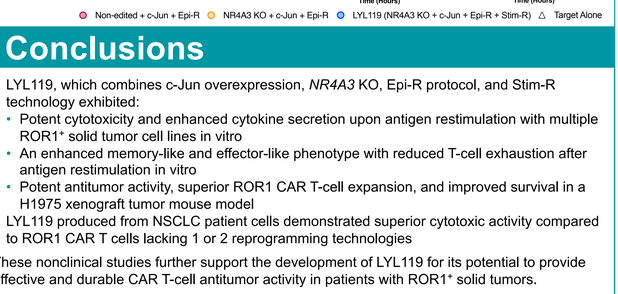


(A) Tumor volume, (B) peripheral blood ROR1 CAR T cells, and (C) animal survival at a 1 x 10^6 and 0.1 x 10^6 CAR T-cell dose range in a H1975 xenograft NSG HLA dKO mouse model. Data from one representative research-scale donor of 3 independent animal studies (n=2 for research and n=1 for clinical scale) with n=6-10 mice/group is shown. Mean tumor volume curves were truncated at timepoints when less than 80% of animals/group remained on study. Error bars represent mean ± SEM. Statistical analysis of peripheral blood ROR1 CAR T cell expansion was performed on Day 21 after T-cell injection. Asterisks indicate significant differences compared to LYL119–treated animals. *p<0.05; **p<0.005; ***p<0.001; ****p<0.0001 by Tukey's mixed-effects one-way ANOVA (A, B) or log-rank Mantel-Cox (C) statistical analyses.

NSCLC patient-derived LYL119 products demonstrate robust cytotoxicity in vitro

- NSCLC patient-derived LYL119 demonstrated similar T-cell fold expansion as healthy donor-derived LYL119 during production
- Approximately 90% *NR4A3* genomic editing efficiency was achieved in NSCLC patient-derived LYL119 products
- NSCLC patient-derived LYL119 demonstrated superior cytotoxicity compared to patient ROR1 CAR T cells lacking 1 or 2 reprogramming technologies (**Figure 7**)

Figure 7: NSCLC patient-derived LYL119 demonstrates superior cytotoxicity in vitro



(A) Fold expansion of T-cell numbers during production calculated as ((total cell count at harvest) / (total cell count activated)). (B) *NR4A3* genomic editing efficiency. Symbols correspond to ROR1 CAR T cells derived from independent donors (n=4 for NSCLC and n=2 for healthy donors). (C) Normalized target killing following sequential stimulation with H1975-NLR NSCLC tumor cells at a 1:25 E:T ratio. Lysis of NLR-expressing tumor cells was quantified by measuring total NLR intensity and normalized relative to the starting intensity for each round of stimulation.

Conclusions

- LYL119, which combines c-Jun overexpression, *NR4A3* KO, Epi-R protocol, and Stim-R technology exhibited:
  - Potent cytotoxicity and enhanced cytokine secretion upon antigen restimulation with multiple ROR1+ solid tumor cell lines in vitro
  - An enhanced memory-like and effector-like phenotype with reduced T-cell exhaustion after antigen restimulation in vitro
  - Potent antitumor activity, superior ROR1 CAR T-cell expansion, and improved survival in a H1975 xenograft tumor mouse model
- LYL119 produced from NSCLC patient cells demonstrated superior cytotoxic activity compared to ROR1 CAR T cells lacking 1 or 2 reprogramming technologies

These nonclinical studies further support the development of LYL119 for its potential to provide effective and durable CAR T-cell antitumor activity in patients with ROR1+ solid tumors.

Methods

- Healthy donor ROR1 CAR T cells were manufactured at research or clinical scale using the Epi-R protocol, activated with Stim-R technology or a standard reagent, and transduced with a lentiviral vector encoding c-Jun, the ROR1 CAR, and an optimized variant of EGFR (EGFRopt). NSCLC patient donor ROR1 CAR T cells were manufactured at research scale.
- T cells were electroporated with a single guide RNA targeting human *NR4A3* or control *CD19* (not expressed in T cells) complexed with SpyFi™ Cas9 nuclease (Aldevron®)
- ROR1 CAR T-cell cytotoxicity, cytokine production, and phenotype were evaluated in vitro following antigen restimulation assays designed to promote T-cell exhaustion
- Antitumor activity of ROR1 CAR T cells was evaluated in vivo using a ROR1-expressing H1975 human NSCLC xenograft model in NSG HLA dKO mice

Abbreviations

4-1BB, tumor necrosis factor ligand superfamily member 9; CAR, chimeric antigen receptor; CD, cluster of differentiation; dKO, double knockout; EGFRopt, optimized variant of epidermal growth factor receptor; E:T, effector-to-target; GNLY, granulysin; HLA, human leukocyte antigen; IFN-γ, interferon gamma; IL-2, interleukin 2; KO, knockout; NR4A3, nuclear receptor subfamily 4 group A member 3; NLR, NucLight Red; NSCLC, non-small cell lung cancer; NSG, NOD scid gamma; ROR1, receptor tyrosine kinase-like orphan receptor 1; scFv, single-chain variable fragment; SD, standard deviation; SEM, standard error of the mean; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3; TNBC, triple negative breast cancer

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